CHAPTER TWO

2.0 GENERAL CHEMICAL ASPECTS OF APOCYNACEAE

Apocynaceae are known to contain a myriad of indole alkaloids with various types of skeletons. In this chapter, the pharmacological activity, biogenesis of indole alkaloid and its synthesis will be discussed briefly, together with the list of all the alkaloids which have been isolated in the *Kopsia* species. The general methods used to elucidate alkaloid structures were also discussed.

2.1 ALKALOIDS

The beginning of alkaloid chemistry was dated back about 180 years ago when F. W. Serturner [5] declared the isolation of morphine **6**. The term "alkaloids" refers to a group of compounds that occur almost exclusively in plants which contain at least one nitrogen atom and constitute part of a heterocyclic ring. Many alkaloids possess therapeutic values, but some are used as flavoring, poison and model compounds for pharmacological studies. A particular alkaloid is customarily restrained to certain genera and families of the plant kingdom [6]. The elaboration of alkaloid is not localised in specific organs. In the perennials, during the first year of the plant growth, the alkaloids seem to be evenly distributed amongst the various organs. However, with increasing age, there appears to be a localisation in a few organs. Thus, in trees, the bark usually becomes denser in alkaloids than the leaves and shoots, owing to accumulation year after year [7, 8]. In the case of annuals, the period of maximum output seems to coincide with the flowering stage [8]. When plant elaborate more than one alkaloid, their ratio need not necessarily be the same at all stages of growth [7]. Cultural and climatic conditions have only a moderate effect on the alkaloid content of the plant [7].

The Apocynaceae family contains largely of indole alkaloids. Until now, more than 2000 indole alkaloids have been discovered.

Even though many alkaloids have been found to exert some type of pharmacological activity, their function within a plant is still a question that has yet to be answered. Many authorities regarded them as by-products of plant metabolism. However, a few suggestions have been voiced by scientists;

a) Alkaloid may act as growth regulators or stimulants,

b) being basic, alkaloid may be involved in maintaining ionic balance in plants,

c) they may provide modest protection on the plant against pests,

d) they may act as reservoir for protein synthesis, and

e) as detoxicating agents of substances whose accumulation might otherwise cause damaged to the plants.

2.2 PHARMACOLOGICAL ACTIVITY

Before their recognition as useful therapeutic agents, alkaloids were renowned for their poisonous properties. Of the many biologically active indole alkaloids, only a few were used today as therapeutic agents of value in human medicine. For example, the treatment of Hodgkin's disease uses vincaleukoblastine **4** with the majority of treated patients showing complete tumor regression or remission and leurocristine **5** was used to treat acute leukemia [1]. Meanwhile, vincamine **2**, an eburnane type alkaloid, has been shown to increase cerebral blood flow in both man [9] and animals [10]. It is also believed that vincamine stimulates neuronal metabolism [11] and apparently improved the associated neurological symptoms of geriatric patients suffering advanced cerebral arteriosclerosis [12]. Vindoline **3**, an aspidosperma alkaloid has been found to have a

weak hypoglycemic activity [13]. Some of the typical effects of reserpine **1** are tranquillization, hypotension, bradycardia, myosis, ptosis, hypothermia, increased gastrointestinal motility, increased rate of defecation [14] and it is most valuable in the treatment of certain types of hypertension, both by lowering blood pressure and relieving tension [16]. The Ergot alkaloid, ergometrine **7** has a direct action on the contraction of uterine muscles.

Some indole alkaloids have totally opposite biological activities even though they are of the same genus [3]. For example, the Strychnos alkaloids, strychnine **8**, acts as a muscle contractor, while toxiferine **9** causes muscle relaxation.

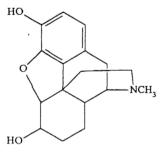
2.3 BIOGENESIS OF INDOLE ALKALOIDS

Mevalonic acid **12**, in conjuction with tryptophan **14**, acts as a precursor to the complex indole alkaloids. There are two very different methods by which mevalonic acid is incorporated into the indole alkaloids.

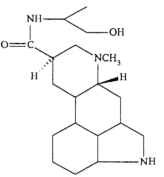
In the biosynthesis of Ergot alkaloids [17], mevalonic acid is inserted as dimethylallyl pyrophosphate **13**. The sequence is shown in Scheme 2.1. 4-isopentyltryptamine **15** is presumed to be an intermediate in this pathway to form lysergic acid, **16**.

However, the majority of indole bases contains tryptamine **17** as the indole nucleus and a C9- or C10- monoterpene moiety, derived from secologanin [3] **18.** Secologanin is made up of two molecules of mevalonic acid.

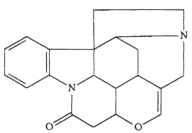
Condensation of secologanin with tryptamine leads to strictosidine **19**, a vincosan skeleton alkaloid (Scheme 2.2). Hydrolysis of the sugar residue and the opening of the cyclic acetal function gives the dialdehyde **20**. Ring closure of **20** yields the tetracyclic system **21**. Minor rearranggements generates ajmalicine **22**, a corynane type alkaloid.



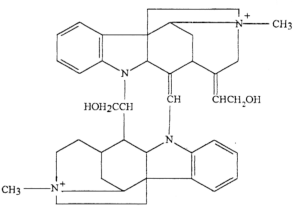
6



7

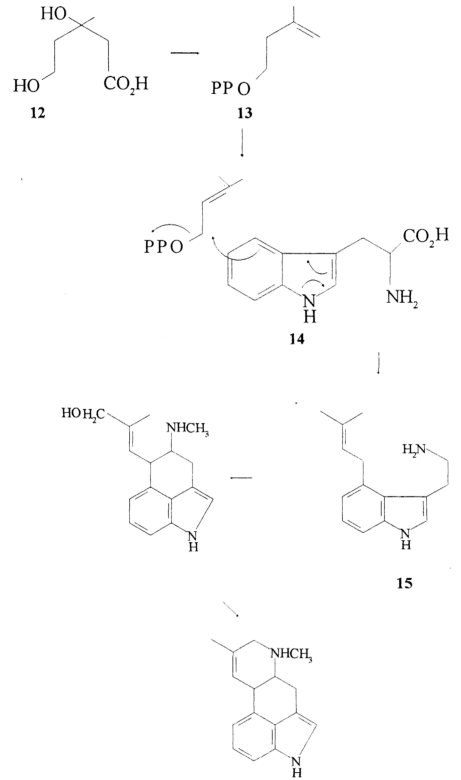


8



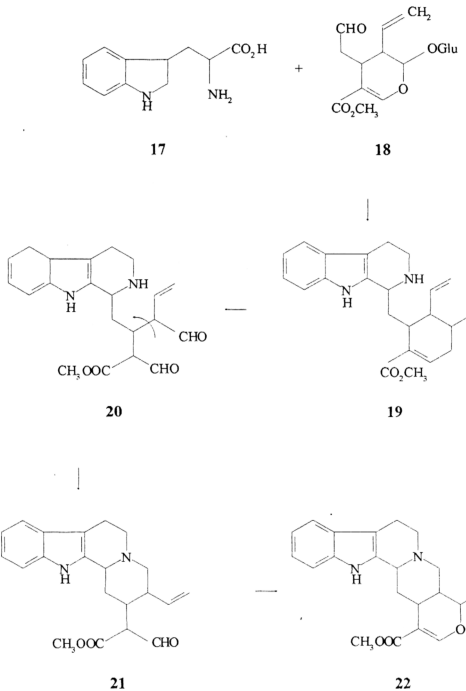
9

Figure 2.1: Examples of indole alkaloids



12 **13** **14** **15** **16**

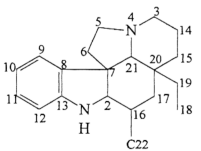
Scheme 2.1 : Biosynthesis of ergot alkaloids



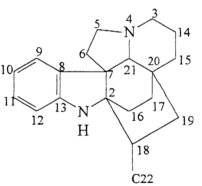
Scheme 2.2 : Condensation of secologanin with tryptamine

2.4 ALKALOIDS FROM *KOPSIA*

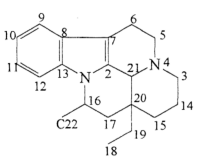
Interest in the chemistry of *Kopsia* species has been directed mostly towards their alkaloids. These alkaloids was first noticed by Greshoff [21] in 1890, who worked on the seeds of *K. flavida* Bl., *K. arborea* Bl. and *K. fruticosa* A. DC. The *Kopsia* genus have species containing mainly alkaloids derived from the aspidospermane type which contained an ethyl chain, the aspidofractinine type whereby C_{18} is linked to C_2. Some also possessed the eburnane skeleton in which a linkage of N_1-C_{16} is observed [22]. All alkaloids which have been isolated from *Kopsia* species are shown in Table 2.1. The structures of these alkaloids are illustrated in Figure 2.2.



aspidospermane type



aspidofractinine type



eburnane type

Table 2.1 : Alkaloids from *Kopsia* species

Species		Isolated alkaloids	Ref.
K.arborea	**30**	(-) kopsinine	23, 24
	31	(-) kopsilongine	
	32	(-) kopsamine	25
	33	(-) kopsiflorine	
	34	methyl N_1-decarbomethoxychanofruticosinate (alk. B)	26
	35	methyl 11,12-methylenedioxychanofruticosinate (alk.C)	
	36	methyl 11,12-methylenedioxy- N_1-decarbomethoxychanofruticosinate	
	37	methyl 11,12-methylenedioxy- N_1-decarbomethoxychano $\Delta^{14,15}$ fruticosinate	
K. dasyrachis	**38**	kopsidasine	27, 28
	39	kopsidasinine	
	40	kopsidasine-N-oxide	
	41	kopsirachine	
K. deverrei	**42**	(-)-N-methoxycarbonyl 17β-hydroxy kopsinine	29,
	43	(-)-N-methoxycarbonyl 17β-hydroxy $\Delta^{14,15}$ kopsinine	30, 22
	44	(+) kopsinone	
	45	(+)-12-methoxykopsinone	
	46	(-)-10-methoxykopsinone	
	47	(-)-14,15-dihydro-10-methoxykopsinone	
	48	pleiocarpamine	
	49	deacetylakuammiline	
	50	(+)-16-hydroxymethylpleiocarpamine	
	51	(+)-16-epi-deacethylakuammiline	
	52	(+)-14α-hydroxycodylocarpine	
	53	(+)-N-methoxycarbonyl-12-methoxykopsinaline	
	32	(+)-N-methoxycarbonyl 11,12-methylenedioxykopsinaline	

K fruticosa	**7**	(-)-kopsine	31,32,
	54	(-)-fruticosine	20, 33
	55	(-)-fruticosamine	
K. griffithii	**31**	kopsilongine	
	32	kopsamine	
	113	kopsamine-N_4-oxide	
	112	pleiocarpine	
	30	kopsinine	
	27	(+)-eburnamonine	
	118	N-methoxycarbonyl 12-methoxy $\Delta^{16,17}$ -kopsinine	
	114	N-methoxycarbonyl 11-hydroxy 12-methoxykopsinaline	
	107	(-)-N-methoxycarbonyl 11,12-dimethoxykopsinaline	
	104	(-)-tetrahydroalstonine	
	135	rhazimol	
	150	harmane	
	151	leuconolam	
	152	buchtienine	
	153	12-methoxykopsidasinine	
	154	16-(R)-19,20-E-isositsirikine	
	155	leuconoxine	
	156	12-methoxypleiocarpine	
	157	harmicine	
K. hainanensis	**30**	(-)-kopsinine	34
	56	(-)-kopsininic acid	
	57	(-)-kopsinoline	
	58	kopsinilam	
	59	kopsanone	
	60	(+)-5,22-dioxokopsane	
	25	eburnamenine	
	26	(+)-eburnamine	
	61	(-)-isoeburnamine	
	62	(+)-tubotaiwine	

	63	(+)-kopsoffine	
K. jasminiflora	**64**	(-)-kopsijasminilam	35, 36
	65	(-)-20-deoxykopsijasminilam	
	66	14,15-dehydrokopsijasminilam	
	67	(-)-kopsijasmine	
	68	(-)-jasminiflorine	
	54	fruticosine	
	55	fruticosamine	
	69	10-demethoxykopsidasinine	
K. lapidilecta	**70**	(-)-lapidilectine A	37, 38
	71	(+)-lapidilectine B	
	72	isolapidilectine A	
	73	lapidilectinol	
	74	isolapidilectinol	
	75	lapidilectone	
	30	kopsinine	
	76	venalstonine	
K. larutensis	**27**	(-)-eburnamonine	39
	26	(-)-eburnamine	
	61	(+)-isoeburnamine	
	30	(-)-kopsinine	
	77	(+)-larutensine	
	78	(-)-eburnaminol	
	93	eburnamine N(4)-oxide	40
K. macrophylla	**79**	8-hydroxyskytanthine	22
	80	8-oxoskytanthine	
	81	$\Delta^{5,9}$ 8-oxoskytanthine	
	82	kopsilactone	
	83	kopsone	
	60	5,22-dioxokopsane	
	84	11,12-metylenedioxykopsinaline	
	85	11,12-metylenedioxy 16-deoxykopsinaline	

	86	dregamine	
	87	tabernaemontanine	
	88	(-)-norpleiomutine	
K. pauciflora	**88**	(-)-norpleiomutine	41, 22
	89	(-)-demetylnorpleiomutine	
	90	(+)-kopsoffinol	
	63	(+)-kopsoffine	
	26	eburnamine	
	91	$\Delta^{14,15}$ -eburnamine	
	61	isoeburnamine	
	92	19-hydroxyeburnamine	
	30	kopsinine	
	58	kopsinilam	
	93	N-methoxycarbonyl 11-methoxy 12-hydroxykopsinaline	
	94	pauciflorine A	42
	95	pauciflorine B	
	96	paucidactine A	43
	97	paucidactine B	
	98	kinabalurine A	44
	99	kinabalurine B	
	100	kinabalurine C	
	101	kinabalurine D	
	102	kinabalurine E	
	103	kinabalurine F	
K. pitardii @	**30**	(-)-kopsinine	45,
K.officinalis	**60**	(+)-5,22-dioxokopsane	46,
	104	(-)-tetrahydroalstonine	47,
	105	(-)-quebrachamine	48,
	150	alkaloid A	49
	34	alkaloid B	
	35	alkaloid C	

61 (-)-isoeburnamine

106 (-)-12-methoxykopsinaline

84 (-)-11,12-methylenedioxykopsinaline

53 (-)-N-methoxycarbonyl 12-methoxykopsinaline

32 (-)-N-methoxycarbonyl 11,12-methylenedioxykopsinaline

107 (-)-N-methoxycarbonyl 11,12-dimethoxykopsinaline

61 (-)-isoeburnamine

63 (+)-kopsoffine

30 kopsinine

30 kopsinine

32 kopsamine

26 eburnamine

108 perivine

109 19-oxo-eburnamine

110 19-oxo-eburnamenine

111 19-hydroxyeburnamine

$C_{32}H_{28}N_2O_2$

$C_{20}H_{20}N_2O_2$

$C_{40}H_{48}N_4O_2$

30 kopsinine

58 kopsinilam

59 kopsanone

60 5,22-dioxokopsane

25 eburnamenine

112 pleiocarpine

32 kopsamine

113 kopsamine-N-oxide

53 N-methoxycarbonyl 12-methoxykopsinaline

107 N-methoxycarbonyl 11,12-dimethoxykopsinaline

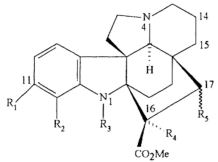
114 N-methoxycarbonyl 11-hydroxy 12-methoxykopsinaline

	115	N-methoxycarbonyl 11-methoxy 12-hydroxykopsinaline	
	116	(+)-vincadifformine	
K. profunda	**117**	N-methoxycarbonyl 11,12-methylenedioxy $\Delta^{16,17}$ -kopsinine	50
	118	N-methoxycarbonyl 12-methoxy $\Delta^{16,17}$ -kopsinine	
K. singapurensis	**119**	(+)-kopsaporine	51,
	120	(+)-kopsingine	52,
		kopsingarine ($C_{23}H_{26\text{-}30}N_2O_7$)	53,
	121	(-)-rhazinilam	54,
	122	(-)-5,21-dihydrorhazinilam	55, 56
	123	akuammidine	
	124	akuammiline	
	125	11,12-methylenedioxykopsaporine	
	126	singapurensine A	57
	127	singapurensine B	
	128	singapurensine C	
	129	singapurensine D	
K. sleesenia	**120**	kopsingine	22
K. teoi	**61**	isoeburnamine	58
	120	kopsingine	
	130	lonicerine	
	121	rhazinilam	
	131	16-epi-17a-hydroxy- ^{14,15} -kopsinine	
	125	11,12-methylenedioxykopsaporine	
	132	kopsidine A	59,
	133	kopsidine B	60,
	134	kopsinginine	61,
	119	kopsaporine	62, 63
	135	rhazimol	
	124	akuammiline	
	136	kopsinol	

	137 kopsinginol	
	138 kopsinganol	
	139 17α-hydroxy-$\Delta^{14,15}$-kopsinine	
	140 kopsinitarine A	
	141 kopsinitarine B	
	142 kopsinitarine C	
	143 nitaphylline	
K. tenuis	**144** lundurine A	64
	145 lundurine B	
	146 lundurine C	
	147 tenuisine A	65
	148 tenuisine B	
	149 tenuisine C	

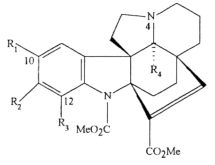
Figure 2.2: Structures of the isolated alkaloids from Kopsia species

Type aspidofractinine

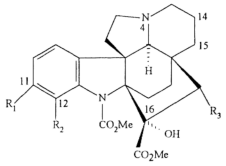


R_1	R_2	R_3	R_4	R_5		
H	H	H	H	H, 5 oxo	Kopsinilam	**58**
H	H	CO_2Me	H	OH	N-methoxycarbonyl 17β-hydroxykopsinine	**42**
H	H	CO_2Me	H	OH, $\Delta^{14,15}$	N-methoxycarbonyl 17β-hydroxy-$\Delta^{14,15}$ kopsinine	**43**
OCH_2O		H	OH	H	11, 12-methylenedioxykopsinaline	**84**
H	OMe	H	OH	H	12-methoxykopsinaline	**106**
H	H	H	H	αOH, $\Delta^{4,15}$	17β-hydroxy-$\Delta^{14,15}$ kopsinine	**139**
H	H	H	H	αOH, $\Delta^{14,15}$	16-epi-17β-hydroxy-$\Delta^{14,15}$ kopsinine	**131**
H	H	H	H	H	kopsinine	**30**
OCH_2O		H	H	H	11, 12-methylenedioxy 16-deoxykopsinaline	**85**
H	H	CO_2Me	H	H	pleiocarpine	**112**
H	OMe	CO_2Me	H	H	12-methoxypleiocarpine	**156**

Type aspidofractinine (continued)

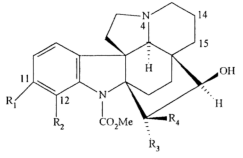


R_1	R_2	R_3	R_4		
OMe	H	H	OH	kopsidasine	**38**
OMe	H	H	OH, N_4-O	kopsidasine N-oxide	**40**
H	H	H	H	kopsijasmine	**67**
H	OCH_2O		H	N-methoxycarbonyl 11,12-methylenedioxy $\Delta^{16,17}$-kopsinine	**117**
H	H	OMe	H	N-methoxycarbonyl 12- methoxy $\Delta^{16,17}$-kopsinine	**118**

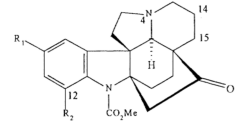


R_1	R_2	R_3		
H	H	OMe	Kopsilongine	**31**
H	OMe	H	N-methoxycarbonyl 12-methoxykopsinaline	**53**
OCH_2O		H	kopsamine	**32**
OCH_2O		H, N_4-O	kopsamine N-oxide	**113**
OMe	OMe	H	N-methoxycarbonyl 11, 12-dimethoxykopsinaline	**107**
OH	OMe	H	N-methoxycarbonyl 11-hydroxy-12-methoxykopsinaline	**114**
OMe	OH	H	N-methoxycarbonyl 11-methoxy-12-hydroxykopsinaline	**115**
H	OH	OH, $\Delta^{14,15}$	kopsaporine	**119**
H	OMe	OH, $\Delta^{14,15}$	12-methoxykopsaporine (kopsingine)	**120**
OCH_2O	OCH_2O	OH, $\Delta^{14,15}$	11, 12-methylenedioxykopsaporine	**125**
H	H	H	kopsiflorine	**33**
H	H		kopsinginine	**134**

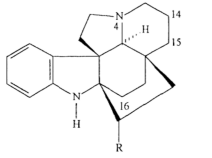
Type aspidofractinine (continued)



R_1	R_2	R_3	R_4		
H	H	CO_2Me	OH	kopsinol	**136**
H	H	H	H, $\Delta^{14,15}$	kopsinginol	**137**
H	OMe	OH	CO_2Me, 15 -αOH	kopsinganol	**138**

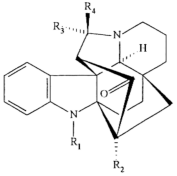


R_1	R_2		
H	H, $\Delta^{14,15}$	(+)-kopsinone	**44**
H	OMe	(+)-12-methoxykopsinone	**45**
OMe	H, $\Delta^{14,15}$	(+)-10-methoxykopsinone	**46**
OMe	H	(-)-14, 15-dihydro 10-methoxykopsinone	**47**

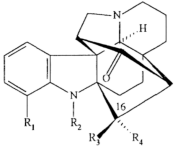


R=COOH, N_4-O	kopsinoline	**57**
R=CO_2Me, $\Delta^{14,15}$	venalstonine	**76**

Type aspidofractinine (continued)

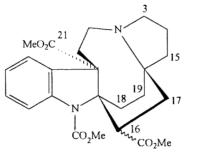


R_1	R_2	R_3	R_4		
CO_2Me	OH	H	H	(-)-kopsine	**7**
H	H	H	H	kopsanone	**59**
H	H	O		(+)-5,22-dioxokopsane	**60**

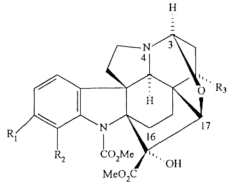


R_1	R_2	R_3	R_4		
H	CO_2Me	OH	H	(-)-fruticosine	**54**
H	CO_2Me	H	OH	(-)-fruticosamine	**55**
OMe	H	OH	H	(-)-jasminiflorine	**68**

Type aspidofractinine (continued)

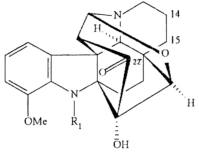


16 α-CO_2Me,$\Delta^{14,15}$	(-)-lapidilectine A	**70**
16 β-CO_2Me, $\Delta^{14,15}$	isolapidilectine	**72**
16 α-CO_2Me, 15 β-OH	lapidilectinol	**73**
16 α-CO_2Me, 15 α-OH	epilapidilectinol	**74**
16 α-CO_2Me, 3-keto, $\Delta^{14,15}$	lapidilectam	**75**



R_1	R_2	R_3		
H	H	OH	singapurensine A	**126**
H	H	OMe	singapurensine B	**127**
OCH_2O		OH	singapurensine C	**128**
OCH_2O		OMe	singapurensine D	**129**
H	OMe	OMe	kopsidine A	**132**
H	OMe	OEt	kopsidine B	**133**

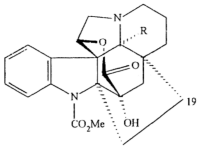
Type aspidofractinine (continued)



R= CO_2Me, $\Delta^{14,15}$ kopsinitarine A **140**

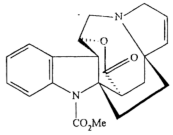
R=H, $\Delta^{14,15}$ kopsinitarine B **141**

R=H, 15-OH kopsinitarine C **142**



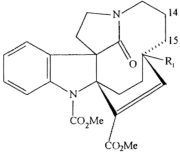
R=OH paucidactine A **96**

R=H paucidactine B **97**



(+)-lapidilectine B **71**

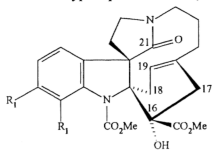
Type aspidofractinine (continued)



R_1=OH kopsijasminilam **64**

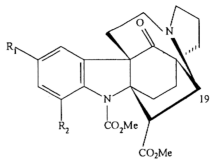
R_1=H 20-deoxykopsijasminilam **65**

R_1=OH, $\Delta^{14,15}$ 14,15-dehydrokopsijasminilam **66**



R_1= R_2=OCH_2O pauciflorine A **94**

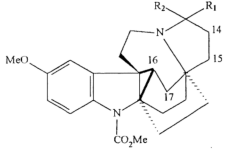
R_1= R_2=OMe pauciflorine B **95**



R_1=OMe, R_2=H kopsidasinine **39**

R_1=H, R_2=H 10-demethoxykopsidasinine **69**

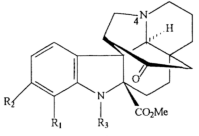
R_1=H, R_2=OMe 12-methoxykopsidasinine **153**



R_1, R_2=O, $\Delta^{14,15}$ lundurine A **144**

R_1= R_2=H, $\Delta^{14,15}$ lundurine B **145**

R_1= R_2=H lundurine C **146**



R_1= R_2=H, R_3=CO_2Me alkaloid A **150**

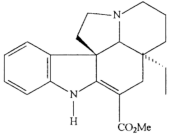
R_1= R_2= R_3=H alkaloid B **34**

R_1= R_2=OCH_2O, R_3=CO_2Me alkaloid C **35**

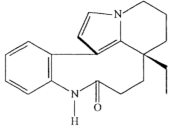
R_1= R_2=OCH_2O, R_3=H methyl 11,12-methylenedioxy-N_1-decarbomethoxy-chanofruticosinate **36**

R_1= R_2=OCH_2O, R_3=H, $\Delta^{14,15}$ methyl 11,12-methylenedioxy-N_1-decarbomethoxy-$\Delta^{14,15}$ chanofruticosinate **37**

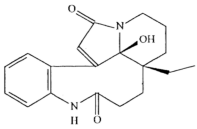
Type aspidospermane



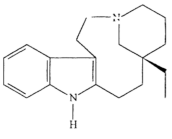
vincadifformine **116**



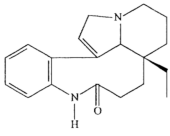
(-)-rhazinilam **121**



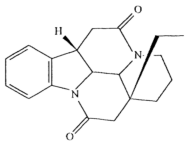
leuconolam **151**



(-)-quebrachamine **105**

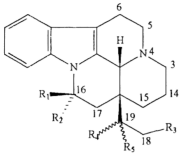


(-)-5,21-dihydrorhazinilam **122**

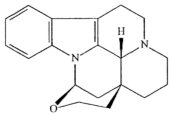


leuconoxine **155**

Type eburnane

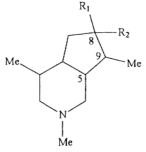


R_1	R_2	R_3	R_4	R_5		
H	OH	H	H	H	eburnamine	**26**
OH	H	H	H	H	isoeburnamine	**61**
OH	H	OH	H	H	eburnaminol	**78**
H	OH	H	H	H, $\Delta^{14,15}$	$\Delta^{14,15}$-eburnamine	**91**
	H	H	H	H, $\Delta^{16,17}$	eburnamenine	**25**
	O	H	H	H	eburnamonine	**27**
	O	H	H	H, N_4-O	eburnamonine N(4)-oxide	**93**
	H	H		O, $\Delta^{16,17}$	19-oxo-eburnamenine	**109**
H	OH	H		O	19-oxo-eburnamine	**110**
H	OH	H	OH	H	19-hydroxyeburnamine	**111**



larutensine **77**

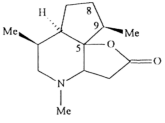
Type piperidine



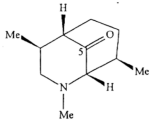
$R_1 = R_2 = OH$ 8-hydroxyskytanthine **80**

$R_1 = R_2 = O$ 8-oxoskytanthine **80**

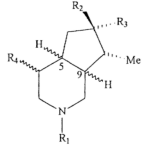
$R_1 = R_2 = O$, $\Delta^{5,9}$ $\Delta^{5,9}$ 8-oxoskytanthine **81**



kopsilactone **82**

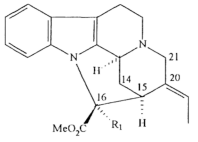


kopsone **83**



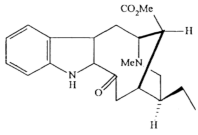
R_1= Me, R_2=OH, R_3=H, R_4= αMe, 5-βH, 9-αH	kinabalurine A	**98**
R_1= Me, R_2 R_3=O, R_4= αMe, 5-βH, 9-αH	kinabalurine B	**99**
R_1= H, R_2R_3=O, R_4=αMe, 5-βH, 9-αH	kinabalurine C	**100**
R_1= Me, R_2=OH, R_3=H, R_4= βMe, 5-αH, 9-βH	kinabalurine D	**101**
R_1= Me, R_2R_3=O, R_4= βMe, 5-αH, 9-βH	kinabalurine E	**102**
R_1= Me, R_2=OH, R_3=H, R_4= αMe, 5-αH, 9-βH	kinabalurine F	**103**

Type corynane



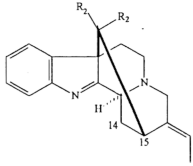
R_1=H (+)-pleiocarpamine **48**

R_1=CH_2OH (+)-16 hydroxymethylpleiocarpamine **50**



dregamine **86**

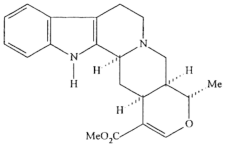
20-epi : tabernaemontanine **87**



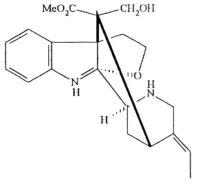
R_1=CH_2OH, R_2=CO_2Me deacetylakuammiline **49**

R_1=CO_2Me, R_2=CH_2OH (+)-16-epi-deacetylakuammiline **51**

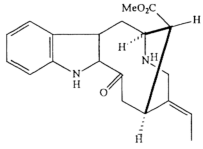
R_1=CH_2OAc, R_2=CO_2Me akuammiline **124**



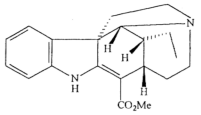
tetrahydroalstonine **104**



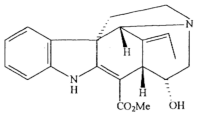
lonicerine **130**



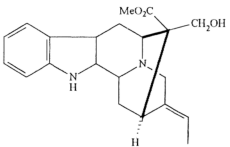
perivine **108**



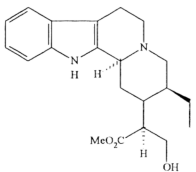
(+)-tubotaiwine **62**



(+)-14 α-hydroxycondylocarpine **52**

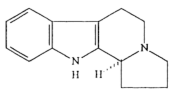


akuammidine **123**



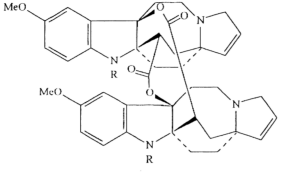
16-(R)-19, 20-*E*-isositrikine **154**

Type harman

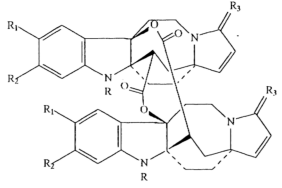


harmicine **157**

Type dimer

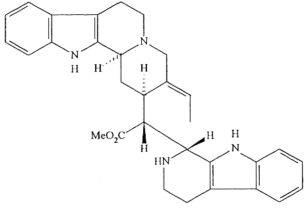


R=CO_2Me tenuisine A **147**

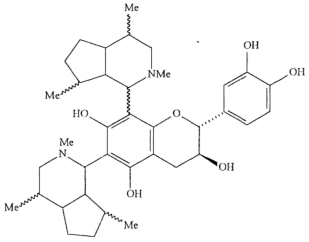


R=CO_2Me, R_1= R_2=OMe, R_3=H_2 tenuisine B **148**

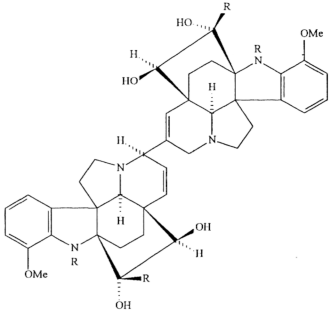
R=CO_2Me, R_1= OMe, R_2= R_3=H_2 tenuisine C **149**



buchtienine **152**



kopsirachine **41**

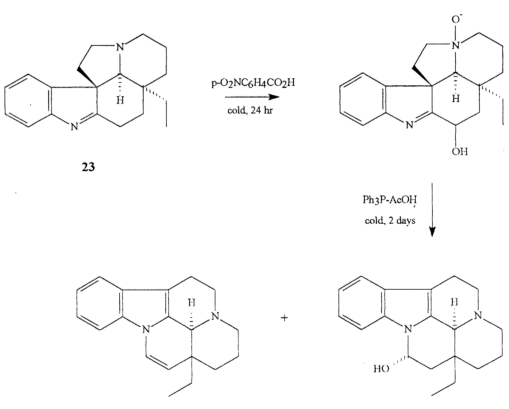


nitaphylline **143**

2.5 STRUCTURAL RELATIONSHIP BETWEEN ASPIDOSPERMANE, ASPIDOFRACTININE AND EBURNANE ALKALOIDS

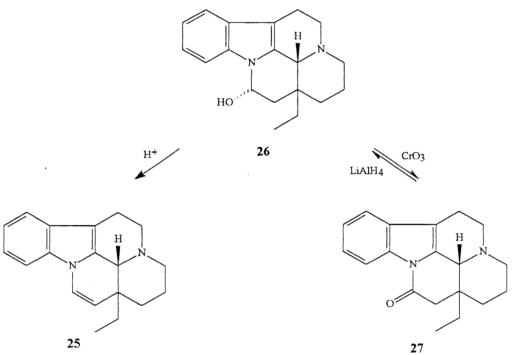
Since the *Kopsia* species possessed mainly alkaloids of the aspidopsermane, aspidofractinine and eburnane skeleton, the structural relationship through synthetic means of these alkaloids were discussed in the following paragraph.

Rearrangement of an aspidospermane skeleton to an eburnane skeleton was shown in Scheme 2.3. Levy and Le Men have transformed (-)-1, 2 - dehydroaspidospermidine **23**, the synthesis yielded vincanol **24** as the main product along with eburnamenine **25** as its minor product [18]. Meanwhile, the interrelationship of eburnamonine **27**, eburnamine **26** and eburnamenine **25** is illustrated in Scheme 2.4. **26** was converted using acids into eburnamenine **25** and with chromic acid, **26** was transformed into eburnamonine **27**. Reduction of eburnamonine **27** regenerated eburnamine **25** [19].



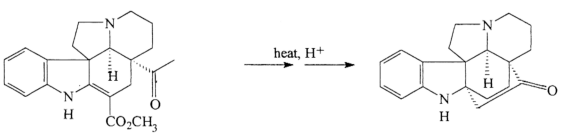
23 **25** **24**

Scheme 2.3 : Rearrangement of an aspidospermane to an eburnane skeleton



Scheme 2.4 : Reactions of eburnamine

It was also shown [20] (Scheme 2.5) that by heating minovincine **28** in acidic medium produces 19-oxoaspidofractinine **29**. Thus the relationship between aspidofractinine and aspidospermane type alkaloid which was found mainly in the *Kopsia* species was established.



28 **29**

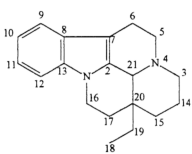
Scheme 2.5: Relationship between aspidospermane and aspidofractinine type alkaloid

2.6 STRUCTURAL ELUCIDATION: GENERAL METHODS AND THEORY

Natural product chemists generally use data collected from nuclear magnetic resonance, ultraviolet, infrared and mass spectroscopy to determine the structure of a molecule. In some cases rotatary dispersion also contributes to its structural identification.

From this study, the author has managed to isolate alkaloids of the eburnane and aspidospermane type, therefore in the following section, the general spectral behaviour of eburnane and aspidospermane alkaloids were discussed briefly.

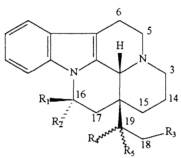
2.6.1 Eburnane



Ultraviolet spectra of eburnanes

The zone of absorption in the UV region for the eburnane skeleton depended upon the substituent type at C-16. Table 2.2 listed the typical values of eburnane UV absorptions. The presence of a hydroxyl and methoxyl group linked to C-16 gave maximum absorptions at 226-229 nm and at 275-280 nm [22, 65]. On the contrary, in eburnamonine **27** and eburnamenine [66] **25**, the molecules revealed a bathochromic shift due to the formation of conjugated molecules, hence all the maximum values were shifted to longer wavelength, for example, eburnamonine **27** revealed absorptions at 301 nm and 310 nm [67]. Also, additional maximum was apparent at 253 nm. As shown

by Table 2.2, modification of the ethyl chain, as in 19-oxo-eburnamine **110**, 19-hydroxyeburnamine **111** and eburnaminol **78** [68] does not seem to present any significant changes in the absorption values compared with eburnamine **26**. This is also true for larutensine **77** that has an ether linkage between C-18 and C-16 [68].



		R_1	R_2	R_3	R_4	R_5	Maximums / nm
eburnamine	**26**	H	OH	H	H	H	229, 276, 282
isoeburnamine	**61**	OH	H	H	H	H	229, 277, 282, 292
eburnaminol	**78**	OH	H	OH	H	H	226, 278, 290
eburnamenine	**25**	H	H	H	H	H, $\Delta^{16,17}$	223, 258, 301
eburnamonine	**27**	O		H	H	H	241, 268, 296, 302
19-oxo-eburnamine	**110**	H	OH	H	O		228, 282, 290
19-hydroxyeburnamine	**111**	H	OH	H	OH	H	229, 282, 292
larutensine	77						226, 278, 290

Table 2.2 : Ultraviolet spectra for eburnanes

Mass spectrum of eburnanes

Alkaloids of the eburnane type give very characteristic fragmentation behaviour. The fragmentations observed from this type of alkaloid are illustrated in Scheme 2.6.

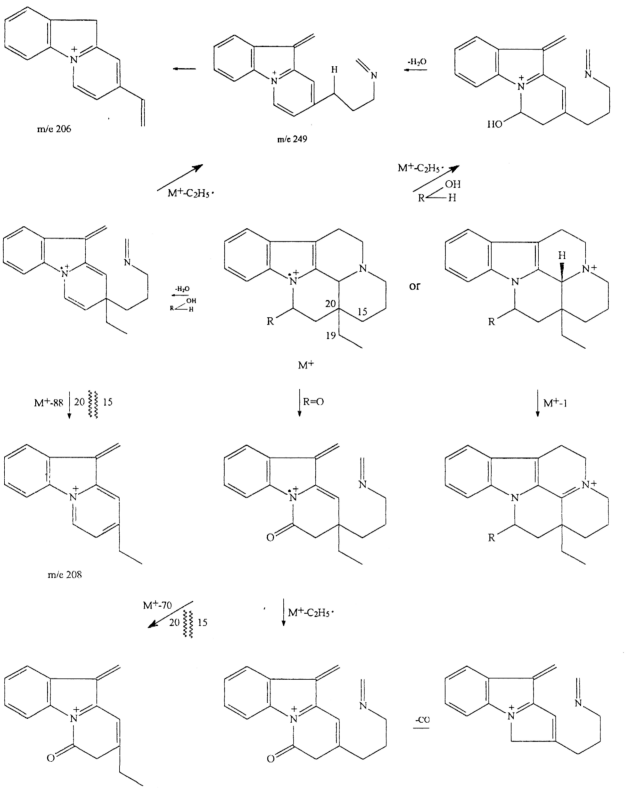
The C20-C15 and C20-C19 allylicaly activated linkages are the most labile bonds in the eburnane molecular ion [69]. Generally, an $[M^+-29]$ peak is detected due to the loss of an ethyl chain. A retro Diels-Alder reaction followed with σ cleavage of the C20-C15

bond by homolysis would give rise to an [M^+-70] fragment for eburnamonine **27** and eburnamenine **25** and an [M^+-88] fragment ion for eburnamine **26** and isoeburnamine **61**[70]. The loss of the ethyl chain was supressed by the effect of an electron withdrawing group like CH_3CO- attached directly to C-16 position thus reducing the intensity of retro Diels-Alder product, ion [M^+-70] as in 19-oxoeburnamine **110**[48]. Thus, an [M^+-43] fragment ion was detected instead of the usual [M^+-29] ion.

^{1}HNMR spectroscopy

Undeniably, the ^{1}HNMR spectra play an important part in determining the structures of compounds. For eburnane alkaloids, several general chemical shifts have been observed.

A singlet for proton H-21 was usually present ranging from δ 3.25 as in larutensine **77** to δ 4.00 in eburnaminol **78**[68]. However, the presence of oxygen at N-4 shifted the H-21 signal further downfield to about δ 4.53 [40]. This was most probably due to the inductive effect of the electronegative oxygen atom that deshielded the H-21 proton. A triplet is detected at δ 0.9 for the methyl in the ethyl chain. Aromatic protons resonated between δ 7.1-7.8 except for eburnamonine **27** whereby its H-12 was shifted further downfield to δ 8.45 due to the electronegative effect of a carbonyl group at C-16. Existence of a hydroxyl group at C-16 deshielded the geminal proton, thus the chemical shift of H-16 ranged from δ 5.5 (eburnamine **26**) to δ 6.00 (isoeburnamine **61**). The configuration of H-16 can be determined by measuring the coupling constant of proton vicinal to H-16, that is H-17 [22, 71]. H-16 in axial position (eburnamine **26**) gave a coupling constant of 10 and 5 Hz, whereas if H-16 was in equatorial position , a small coupling constant, J=4 and 2 Hz was detected (isoeburnamine **61**).



Scheme 2.6: General mass fragmentations for eburnanes

^{13}CNMR spectroscopy

In general, the carbons of eburnane alkaloids resonate at specific regions depending upon the type of hybridization of the carbon.

The resonances are summarized below [41, 72, 73];

sp carbons in the aromatic ring (9, 10, 11, 12): 110 to 125 ppm with C-12 the most shielded.

sp^2 carbons 2, 8, 13: δ 128-138

sp^2 carbons 7: δ 104-108

sp^3 carbons 3, 5: δ 42-54

sp^3 carbons 14, 15, 19: δ 20-30 (However, when C-18 possessed an oxygen atom, for example in eburnaminol **78** and larutensine **77**, the resonances for C-15 and C-19 were shifted further downfield to δ 35 and 40, respectively).

sp^3 carbon 17: δ 38-44

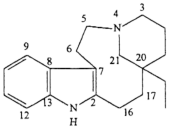
sp^3 carbon 16: δ 75, due to the presence of hydroxyl, methoxyl or ethoxyl group.

sp^3 carbon 18 (CH_3) : δ 6-8

(CH_2OH): δ 58

sp^3 carbon 21: δ 55-63

2.6.2 Aspidospermane



Ultraviolet spectra of aspidospermane

Generally, aspidospermane skeleton with a hydroxyindolenine chromophore have the same absorption maximums as an indole chromophore. These maximums are at 210-227, 282 and 290 nm [74, 75, 76] (refer Table 2.3).

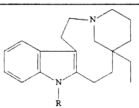
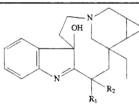
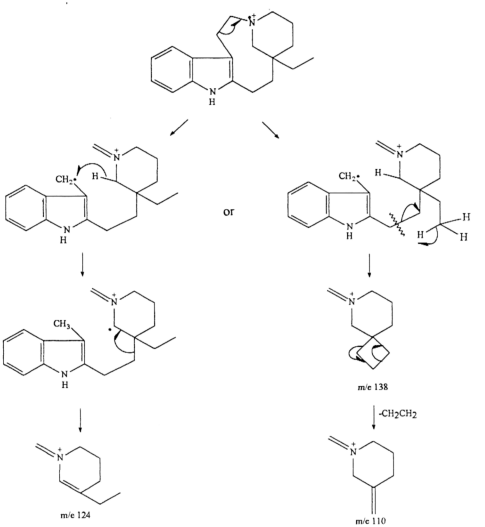
Chromophore	Skeleton	Alkaloid	Maximums/ nm
indole		quebrachamine **105** R=H voafinidine **158** R=Me	227, 284, 291 232, 288, 294
hydroxyindolenine		R_1=R_2=H, R_3=H_2 rhazhidigenine **159** voalenine **160** R_1R_2=O, R_3=O	 227, 282, 290 204, 227, 297

Table 2.3: Ultraviolet spectra for aspidospermanes

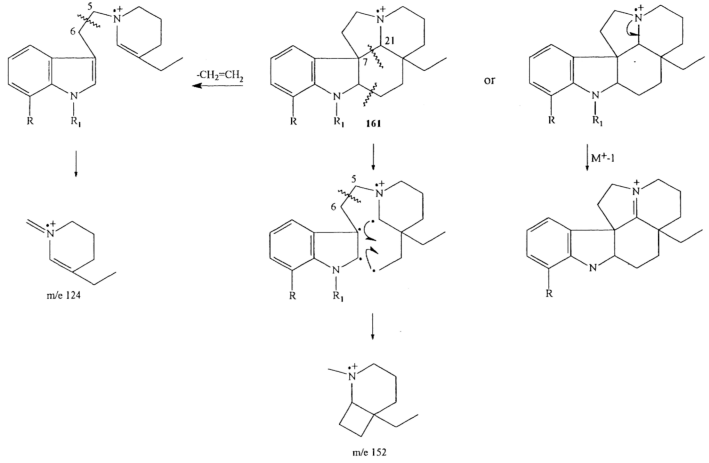
Mass spectrum for aspidospermanes

An intense peak at m/e 124 is a characteristic fragment for aspidospermane alkaloids. Aspidospermane alkaloids which do not have a C21-C7 linkage, for example quebrachamine **105** released a fragment ion M^+-29 due to the loss of the ethyl chain [69]. In addition, a significant fragment at m/e 110 was detected (see Scheme 2.7).

On the contrary,when a linkage existed between C21 and C7, as in aspidospermine **161**, the fragmentation started with a retro Diels-Alder type decomposition and instead of releasing M^+-29, it produced fragment ion M^+-28 which corresponded to the loss of ethylene [77] (see Scheme 2.8). Furthermore, a noticeable M^+-1 peak can be seen due to the expulsion of H-21 [78].



Scheme 2.7: General mass fragmentations for aspidospermane alkaloid without C21-C7 linkage



Scheme 2.8: General mass fragmentations for aspidospermane alkaloid

with C21-C7 linkage

^{1}HNMR spectroscopy

Aspidospermane showed a triplet at *ca* δ 0.9 for the methyl of the ethyl side chain. Without the C21-C7 linkage, H-21 signal was seen as a doublet with a coupling constant of 12 Hz at δ 3.2 (H-21α) and δ 3.6 (H-21β). Aromatic protons resonated as low as δ 6.7 for H-12 (rhazhidigenine **159**)[75] to δ 7.5 for H-9 (quebrachamine **105**)[74].

^{13}CNMR spectroscopy

Below are several general signals for carbons of aspidospermane without the C21-C7 linkage.

sp^2 carbons in the aromatic ring (9, 10, 11, 12): δ 108-131

sp^2 carbons 2, 8, 13: δ 127-141

sp^2 carbon 7: δ 108

sp^3 carbons 3, 5: δ 51-62

sp^3 carbons 14, 15, 19: δ 20-35 (However, with oxygen substitution at C14 and C15, the signal resonated at δ 50-80 [76])

sp^3 carbon 17: δ 32-42

sp^3 carbon 18: δ 7-8

sp^3 carbon 21: δ 53-64